I. Rejection under 35 U.S.C. § 112, First Paragraph - Enablement

The Examiner rejects claims 6-26 under 35 U.S.C. § 112, first paragraph, for allegedly encompassing subject matter that is not sufficiently enabled by the specification. The Examiner alleges that although the specification is enabling for some internal controls, the specification does not provide sufficient enablement for *β-actin* as an internal control. Applicant respectfully disagrees with the Examiner's rejection.

The claims recite a method of determining the relative level of Dihydropyrimidine dehydrogenase (*DPD*) gene expression in a tissue sample comprising; obtaining a tumor sample from a patient; isolating mRNA from the tumor sample; determining the amount of *Dihydropyrimidine Dehydrogenase (DPD)* mRNA by amplifying the mRNA using an oligonucleotide primer having the sequence of SEQ ID: 7, or an oligonucleotide primer at least or about 80% identical therewith and hybridizes to SEQ ID NO: 7 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of Exon 6 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 8, and; an oligonucleotide having the sequence SEQ ID: 8 or an oligonculeotide primer at least or about 80% identical therewith and hybridizes to SEQ ID NO: 8 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of Exon 6 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 7;

and comparing the amount of *Dihydropyrimidine Dehydrogenase (DPD)* mRNA to an amount of mRNA of an internal control.

The claims are also drawn to a method of determining the relative level of Dihydropyrimidine dehydrogenase (*DPD*) gene expression in a tissue sample comprising; obtaining a tumor sample from a patient; isolating mRNA from the tumor sample; determining the amount of *Dihydropyrimidine Dehydrogenase (DPD)* mRNA by amplifying the mRNA using an oligonucleotide primer having the sequence of SEQ ID: 1, or an oligonucleotide primer at least or about 80% identical therewith and hybridizes to SEQ ID NO: 1 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 2, and; an oligonucleotide having the sequence SEQ ID: 2 or an oligonculeotide primer at least or about 80% identical therewith and hybridizes to SEQ ID NO: 2 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 1; and comparing the amount of Dihydropyrimidine dehydrogenase (*DPD*) mRNA to an amount of mRNA of an internal control gene.

The Examiner argues that *β-actin* is not a suitable internal control gene in tumor cells because it is not possible to determine the effects of a patient's individual treatment history on

the gene expression of *β-actin* or other cellular markers. To support his position, the Examiner cites Willhauck *et al.*, BioTechniques 25:656-659 (October 1998). The Examiner quotes Willhauck *et al.* which states, "the increasing number of *GAPDH* and *β-actin* pseudogenes which can be amplified even if mRNA-specific primers were designed, can lead to an overestimation of the RT efficacy." Willhauck *et al.* teaches spiking a blood sample being analyzed for *tyrosinase* gene expression by melanoma cells, with a pre-set number of Jurkat T-cells to act as an internal control.

Additionally, the Examiner cites Selvey *et al.*, Molecular and Cellular Probes, 15: 307-311, (2001). The Examiner quotes Selvey *et al.* as stating: "These results clearly demonstrate the unsuitability of *β-actin* as an internal control for gene expression studies." However, Selvey *et al.* only teaches that the use of *β-actin* is not appropriate as an internal control gene in a "specific case" (page 308, left column) i.e., when assaying the relative expression levels of *MT1-MMP membrane type1 - matrix metalloproteinase (MT1-MMP)* in fibroblasts grown on a coat of matrigel matrix. Selvey *et al.* also states that although there is evidence that *β-actin* may not be appropriate as an internal control gene in all circumstances, it nonetheless is the, "most widely used internal control in molecular biology," and that it, "remains a popular choice for countless RT-PCR applications." (p. 308, left column). In view of their observations with respect to *β-actin* as an internal control gene when assaying the relative expression levels of *MT1-MMP* in fibroblasts grown on a coat of matrigel matrix, Selvey *et al.* suggest that investigators using this gene as an internal control should first, "demonstrate to a satisfactory degree, that it is not

regulated in their specific application." (p. 310, right column).

Applicant respectfully submits that regardless of the Examiner-cite references, one of skill in the art would be more than satisfied that *β-actin* is appropriate as an internal control gene when assaying the relative expression level of *DPD* and other genes in various tissues.

Salonga *et al.*, Clinical Cancer Research, 6:1322-1327, (April 2000) reported that when using *β-actin* as an internal control, "a linearity is obtained between gene expression values determined by RT-PCR and protein content determined by immunohistochemistry." (p. 1323, right column). As such, they reported relative expression levels of *Thymidylate Synthase (TS)* and *DPD* as a ratio of *TS* and *DPD* RT-PCR products to *β-actin* RT-PCR products, respectively, in 33 colorectal tumors. Johnston, *et al.*, Cancer Research, 55(7):1407-12, (April 1995), reported a "close linear relationship between TS protein expression and *TS* gene expression" when using *β-actin* as an internal control gene in 21 tumor samples (9 colorectal and 12 gastric, p. 1409, right column). For illustrative purposes, Johnston and Salonga teach one of ordinary skill in the art that if *TS* gene expression is reported to be 2 times *β-actin* expression in a sample A and 3 times *β-actin* expression in another sample B, that the immunohistochemical TS protein signal in sample B was consistently observed to be 50% greater than in sample A. The consistency of this observation was characterized as a "close linear relationship" and teaches that *β-actin* expression provides a reliable control.

More recently, Gustavsson *et al.*, ASCO Annual Meeting of 2002, Abstract 457, reported measuring relative *TS, DPD, TP*, folypolyglutamate synthetase (*FPGS*) and gamma-glutamyl

hydrolase (*GGH*) mRNA expression levels in paraffin-embedded primary tumor tissues from 26 patients with colorectal cancer using *β-actin* as an internal control gene.

(http://www.asco.org/asco/ascoMainConstructor/1,1003,_28-003-00_12-002326-00_29-00A-00_18-002002-00_19-00457,00.asp).

Additionally, Eads *et al.*, Cancer Research, 59:2302-2306 (May 15, 1999) teach the measurement of several DNA Methyltransferase (*DNMT1, DNMT3A, DNMT3B*) genes' expression relative to *β-actin, RNA polymerase II large subunit* as well as *histone H4* and *PCNA* gene expression in 25 human colorectal adenocarcinoma tumor tissue samples and 25 matching non-tumor mucosal samples. The study determined that *β-actin* and *RNA polymerase II large subunit* genes, considered to be non-proliferation associated genes, were appropriate internal control genes, whereas the proliferation associated genes for *histone H4* and *PCNA* were not. (p. 2304, paragraph bridging right and left columns).

Finally, to further cement the fact that one of ordinary skill in the art would readily accept the suitability of *β-actin* as an internal control gene, Applicant herewith provides a list of dozens of her own peer-reviewed studies reporting the successful use of *β-actin* in a large variety of gene expression and tissue applications. Please see Appendix A.

Moreover, other groups have reported similarly satisfactory results using *GAPDH* as an internal control gene, when analyzing *DPD* expression in various tissues. Takechi *et al.*, Japanese Journal of Cancer Research, 89: 1144-1153 (November 1998) report the relationship between DPD protein levels and *DPD* gene expression relative to *GAPDH* in pancreatic carcinoma and fibrosarcoma cells grown in culture and in nude mice. Similarly, Uetake *et al.*,

Clinical Cancer Research, 5:2836-2839 (October 1999), report the relationship between intratumoral DPD protein activity and *DPD* gene expression relative to *GAPDH* in human colorectal tumor samples and paired non-tumoral tissue samples. Uetake *et al.* state that an internal control gene, such as *GAPDH*, "is expressed in a constant per-cell level," and is useful for normalization of target gene expression. (p. 2837, left column). Finally, Johnson *et al.*, Analytical Biochemistry 278: 175-184 (2000), assayed *DPD* gene expression relative to *GAPDH* in colorectal and liver tumors by Real-Time RT-PCR using a TaqMan® system similar to that exemplified in the subject application. The investigators found that as opposed to the semi-quantitative RT-PCR of the past, the Real-Time RT-PCR methodology utilizing *GAPDH* as an internal control gene is "accurate and reproducible" and has, "low inter- and intra-assay variations." (p. 183, right column). Moreover, they suggest that it might someday even be possible to, "quantitate DPD mRNA in archival material such as paraffin-embedded tissues." (p. 183, left column). The authors point out that this would be advantageous because, "the response to chemotherapy is known," in the archival samples, "correlations between response to 5-FU and DPD mRNA levels could readily be determined." (p. 183, left column). The subject application provides a road map for carrying out such studies by providing a method for determining relative *DPD* expression in a tissue.

In view of these extensive findings, Applicant respectfully asserts that one of ordinary skill in the art would readily accept the utility of *β-actin* and *GAPDH* as suitable internal control genes for determining the relative *Dihydropyrimidine dehydrogenase* (*DPD*) gene expression in a

tissue sample. Again, even if Selvey *et al.* state that although there is evidence that *β-actin* may not be appropriate as an internal control gene in all circumstances, it nonetheless is the, "most widely used internal control in molecular biology," and that it, "remains a popular choice for countless RT-PCR applications." As such, one of skill in the art would know how to make and use the claimed invention without undue experimentation with respect to the claim recitations "internal control gene" and "*β-actin*" as an internal control gene.

Setting aside these peer-reviewed studies, even if one of skill in the art were not to consider *GAPDH* or *β-actin* to be appropriate internal controls genes for purposes of the claims at issue, one of skill in the art would readily and without undue experimentation, chose any other internal control gene they deem appropriate to make and use the claimed invention.

The specification defines internal control genes as including any constitutively or globally expressed gene whose presence enables an assessment of relative *DPD* mRNA levels. The specification further indicates that such internal control genes allow for a determination of the overall constitutive level of gene transcription and control for variations in RNA recovery. As such, one of skill in the art would instantly recognize that any gene satisfying these criteria would be appropriate as an internal control gene. As examples the specification lists the cyclophilin gene, *β-actin* gene, the transferrin receptor gene, *GAPDH* gene, and the like.

Regardless of whether or not a small handful of studies suggest, that *β-actin* and *GAPDH* might be suitable internal control genes for assaying *DPD* expression in tissue samples, the skilled artisan could readily pick any genes such as *cyclophilin, transferrin receptor, 18S RNA,*

Peptidylprolyl isomerase A (PPIA), or any other well known internal control gene.

The Examiner even indicates that the specification is enabling for some internal controls. Unless a particular internal control gene is shown to be clearly inappropriate for detecting a specific gene in from a particular cell-type in a specific context, e.g. when assaying the relative expression levels of MT1-MMP in fibroblasts grown on a coat of matrigel matrix, then for purposes of the claimed methods for determining the relative level of Dihydropyrimidine dehydrogenase (*DPD*) gene expression in a tissue sample, the identity of the internal control gene is irrelevant. As such, because the specification provides enablement for some internal control genes, as indicated by the Examiner, then the Applicant is entitled any internal control that one of skill in the art would consider suitable for a method of determining the relative level of Dihydropyrimidine dehydrogenase (*DPD*) gene expression in a tissue sample. Such a decision requires no undue experimentation given the fact that there are many commercial RT-PCR kits available that supply a reagents for number of internal control genes. Accordingly, Applicant respectfully requests withdrawal of this ground for rejection.

II. Rejection under 35 U.S.C. § 112, First Paragraph - Written Description

The Examiner has rejected claims 6-26 under 35 U.S.C. § 112, first paragraph, as allegedly encompassing subject matter lacking sufficient written description. The Examiner alleges that the specification discloses SEQ ID Nos: 1, 2, 7 and 8, and no specific examples of nucleic acids that are "substantially identical" to them. The Examiner further argues that "substantially identical" encompasses a genus of oligonucleotides that are not described in the

specification and as a result one of ordinary skill in the art would not be convinced that the Applicant was in possession of the claimed genus at the time of filing.

The Examiner refers to *The Regents of the University of California v. Eli Lilly and Co.* 43 U.S.P.Q.2d 1398 (Fed. Cir.1997) as a basis for his Written Description rejection. The Federal Circuit stated:

> ... 'mammalian insulin cDNA,' without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function . . . . It does not define any structural features commonly possessed by members of the genus that distinguish them from others . . . . It is only a definition of a useful result rather than what achieves it.

UC v. Eli Lilly, states that a claim containing only functional limitations without structural characteristics is insufficient to satisfy the Written Description requirement. In order to clarify this requirement, Example 9 of the Written Description Guidelines (Federal Register, Vol. 66, No. 4, Friday, January 5, 2001), states that,

> [A] person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because highly stringent conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, **since highly stringent hybridization conditions *in combination* with the coding function of DNA** and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

The Applicant, respectfully asserts that the amended claims at issue combine a functional recitation and several structural recitations and thereby encompass subject matter sufficiently described in the invention.

To expedite prosecution of the subject application, the claims have been amended to read on isolated and purified oligonucleotides SEQ ID NOs: 1, 2, 7 and 8 and oligonucleotides at least

or about 80% identical therewith and that hybridize to the complements of SEQ ID NOs: 1, 2, 7 and 8, respectively, under stringent conditions; which are further functionally characterized by their capability to amplify a portion of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used in conjunction with a with a second oligonucleotide.

Applicant respectfully points out that the claims at issue do not merely define the invention solely by the function that the oligonucleotides encompassed therein, have the capability to amplifying any portion of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used in conjunction with a with SEQ ID NOs: 1, 2, 7 or 8, respectively. The claims at issue also read on only those oligonucleotides that are structurally defined as least or about 80% identical to those actually reduced to practice. Accordingly, a genus of oligonucleotides that is structurally defined as least or about 80% identical with a hypothetical 20-base oligonucleotide, would differ from the hypothetical oligonucleotide at about 4 positions. This could result in only about 4^4 or approximately 256 specific possibilities. To a person of skill in the art, this does not represent a substantial variation among species encompassed within the scope of the claims, particularly in view of the overarching fact that these approximately 256 species represent merely 2.32×10^{-8} % of the approximately 1-trillion (i.e., 4^{20}) possible 20-base oligonucleotide combinations.

Moreover, the claims also read on oligonucleotides that hybridize to complements of SEQ ID NOs: 1, 2, 7 and 8, respectively, under stringent conditions. The specification defines stringent hybridization conditions as those under which only highly complementary nucleic acid

sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having 4 or more mismatches out of 20 contiguous nucleotides. The hybridizing portion of the nucleic acids is typically at least 10 (e.g., 15) nucleotides in length. The hybridizing portion of the hybridizing nucleic acid is at least about 80% identical to the sequence of a portion or all of oligonucleotide primer SEQ ID NO:1, SEQ ID NO: 2, SEQ ID NO: 7, or SEQ ID NO: 8.

The specification also teaches the parameters for the hybridization of an oligonucleotide primer to a nucleic acid sample under stringent conditions. Nucleic acid duplex or hybrid stability is expressed as a melting temperature (T_m), which is the temperature at which the probe dissociates from the target DNA. This melting temperature is used to define the required stringency conditions. If sequences are to be identified that are at least or about 80% identical to the probe, rather than identical, then it is useful to first establish the lowest temperature at which only holmologous hybridization occurs with a particular concentration of salt (e.g. SSC or SSPE). Then assuming that 1% mismatching results in about 1^0C decrease in T_m, the temperature of the final wash in the hybridization reaction is reduced accordingly. In this case, if sequences having at least or about 80% identity with the probe are sought, the final wash temperature is decreased by at most or about 20^0 C. For example, in order to hybridize a test oligonucleotide to SEQ ID NO: 1 (T_m, = 59^0C) under these stringent conditions, one might select a washing temperature of 39^0C. However, the specification also teaches that in practice, the change in T_m can be between 0.5^0C and 1.5^0C per 1% mismatch.

The specification further teaches exemplary stringent conditions to involve hybridizing in 5x SSC/5x Denhart's solution/1.0% SDS, and washing conditions from about 0.2x SSC/0.1%

SDS to about 3x SSC. Maniatis *et al.*, Molecular Cloning, a laboratory manual, (2nd ed.), Cold Spring Harbor Laboratory Press, New York, (1989) and F. M. Ausubel et al eds., Current Protocols in Molecular Biology, John Wiley and Sons (1994), states that a 20x SSC stock solution contains 175.3g of sodium chloride and 88.2g of sodium citrate in one liter of water, corresponding to approximately 4M sodium ion concentration. Accordingly, the specification discloses stringent hybridization conditions of about 1.0M sodium ion concentration and wash conditions of about 0.01M to about 0.6M sodium ion concentration.

Accordingly, one of skill in the art would know and the specification teaches how parameters of salt concentration and temperature may be fine-tuned to achieve an optimal level of identity between the primer and the target nucleic acid, i.e. at least or about 80%.

Additionally, the claimed oligonucleotides are yet further structurally characterized with respect to the region of the *Dihydropyrimidine Dehydrogenase (DPD)* mRNA they are capable of amplifying. For example, the oligonucleotides recited in claim 6 are capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue. Furthermore, the oligonucleotides recited in claim 17 are capable of amplifying a portion of Exon 6 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue.

When taking these structural characterizations together, the skilled artisan would not expect substantial variation among species encompassed in the scope of the claims because such oligonucleotides are structurally similar DNAs. Thus, the claims are certainly not merely

functionally defined and cannot be read to constitute a "definition of a useful result rather than what achieves it," as put forward by the Examiner in his citation of *U.C. v. Eli Lilly, supra.*

In combination with the structural elements indicated above, the claimed oligonucleotides are yet further characterized by their functional capability to amplify a portion of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used in conjunction with a second disclosed oligonucleotide. Thus, the specification constitutes the disclosure of a representative number of species, since the percentage of identity (i.e. at least or about 80%) as well as the highly stringent hybridization conditions, *in combination* with the amplification function of DNA and the level of skill and knowledge in the art, particularly with respect to the known open reading frame sequence of *DPD*, are adequate to determine that applicant was in possession of the claimed genuses at the time the application was filed. Accordingly, Applicant submits that the application is in condition for allowance because the amendments fully address this rejection. Withdrawal thereof is respectfully requested.

III. Rejection under 35 U.S.C. § 103(a)

The Examiner rejects claims 6-13, 15-24, and 26 under 35 U.S.C. § 103 (a) as allegedly being unpatentably obvious over Gonzales *et al.*, U.S. Patent No. 6,015,673 in view of Willhauck *et al.*, BioTechniques, 1998, 25:655-659. The Examiner opines that SEQ ID NO: 5 of Gonzales *et al.* teaches an oligonucleotide with 14 of 18 nucleotides identical to claimed SEQ ID NO: 1. The Examiner further alleges that such an oligonucleotide is 77% identical with claimed SEQ ID

NO: 1. Applicant respectfully asserts that Examiner is mistaken and not only because the claimed SEQ ID NO: 1 contains 19 nucleotides not 18 (and is therefore not 77% identical).

However, in order to expedite prosecution of the Application, Applicant has amended independent claim 6 to recite a purified oligonucleotide consisting of the sequence of SEQ ID NO: 1, or a sequence which is at least or about 80% identical therewith and hybridizes to a complement of SEQ ID NO: 1 under stringent conditions; wherein the isolated and purified oligonucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 2. As such claim 6 does not read on Gonzales *et al.*

Moreover, independent claim 6 cannot be construed to be unpatentably obvious in view of Gonzales *et al.* As stated in the previous response, amplifying mRNA isolated from fixed and paraffin embedded tissue constitutes a unique challenge due to the level of RNA degradation and chemical modification resulting from the fixation and paraffin-embedding process. For example, extensive RNA cross-linking typically prevents extraction and amplification of RNA stretches greater than 200 bases. As such, it is extremely difficult to identify oligonucleotide pairs suitable for amplification of FPE tissue extracted RNA.

Although it appears that Gonzales *et al.*'s RTF1 and RTR1 are useful for amplifying a 1.5kb *DPD* cDNA fragment, there is nothing in Gonzales *et al.* that would suggest to one of skill in the art that either RTR1 nor the allegedly anticipatory RTF1, either together or individually, would be appropriate for amplifying mRNA isolated from fixed and paraffin embedded tissue. Therefore, one of skill in the art would not be motivated to modify either RTF1 or RTR1, let

alone search for or design a purified oligonucleotide consisting of the sequence of SEQ ID NO: 1, or a sequence which is at least or about 80% identical therewith and hybridizes to a complement of SEQ ID NO: 1 under stringent conditions; wherein the isolated and purified oligonucleotide is capable of amplifying a portion of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 2, as claimed. Accordingly, it can further be concluded that Gonzales *et al.* cannot be asserted to suggest or contemplate the claimed oligonucleotides either.

Additionally, Willhauck *et al.* does not teach any of the remaining claim elements and therefore there exists no motivation for one of skill in the art to combine Willhauck *et al.* and Gonzales *et al.* Moreover, even if one of skill in the art was motivated to draw on the teachings of Willhauck *et al.*, the combination of these references does not render the claims unpatentably obvious because, even together, they do not teach, suggest nor contemplate an oligonucleotide consisting of the sequence of SEQ ID NO: 1, or a sequence which is at least or about 80% identical therewith and hybridizes to SEQ ID NO: 1 under stringent conditions; wherein the isolated and purified oligonucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 2.

In view of the remarks and amendments made herein, Applicant respectfully asserts that the rejection is traversed, and withdrawal thereof is respectfully requested.

CONCLUSION

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 11-0600.

Respectfully submitted,

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Date: Nov. 7, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Please cancel Claims 12-16 and 23-25 without prejudice or disclaimer.

Please amend the claims as indicated:

6. A method of determining the relative level of Dihydropyrimidine dehydrogenase (*DPD*) gene expression in a tissue sample comprising:

(a) obtaining a tumor sample from a patient;

(b) isolating mRNA from said tumor sample;

(c) determining the amount of *Dihydropyrimidine Dehydrogenase (DPD)* mRNA by amplifying the mRNA using an oligonucleotide primer having the sequence of SEQ ID: 1, [or which is substantially identical thereto] or an oligonucleotide primer at least or about 80% identical therewith and hybridizes to a complement of SEQ ID NO: 1 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 2,

and;

an oligonucleotide having the sequence SEQ ID: 2[, or which is substantially identical thereto] or an oligonculeotide primer at least or about 80% identical therewith and hybridizes to a complement of SEQ ID NO: 2 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of the 5' untranslated region and Exon 1 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 1;

(d) comparing the amount of Dihydropyrimidine dehydrogenase (*DPD*) mRNA from step (c) to an amount of mRNA of an internal control gene.

9. The method of claim 8, wherein the tumor sample is embedded in paraffin [fixed] after being fixed.

17. A method of determining the relative level of Dihydropyrimidine dehydrogenase (*DPD*) gene expression in a tissue sample comprising;

(a) obtaining a tumor sample from a patient;

(b) isolating mRNA from said tumor sample;

(c) determining the amount of *Dihydropyrimidine Dehydrogenase (DPD)* mRNA by amplifying the mRNA using an oligonucleotide primer having the sequence of SEQ ID: 7, [or which is substantially identical thereto] or an oligonucleotide primer at least or about 80% identical therewith and hybridizes to a complement of SEQ ID NO: 7 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of Exon 6 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 8,

and;

an oligonucleotide having the sequence SEQ ID: 8[, or which is substantially identical thereto] or an oligonculeotide primer at least or about 80% identical therewith and hybridizes to a complement of SEQ ID NO: 8 under stringent conditions; wherein said isolated and purified oligonucleotide is capable of amplifying a portion of Exon 6 of a *Dihydropyrimidine Dehydrogenase (DPD)* mRNA isolated from fixed and paraffin embedded (FPE) tissue when used with SEQ ID NO: 7;

(d) comparing the amount of the mRNA from step (c) to an amount of mRNA of an internal control.

19. The method of claim 17, wherein the a tumor sample is embedded in paraffin [fixed] after being fixed.

26. The method of any one of claims [5, 6, 12, 17, or 23] 6 or 17; wherein the at least one tissue sample contains bronchoalveolar tumor tissue, small bowel tumor tissue or colon tumor tissue.

APPENDIX A

To further cement the fact that one of ordinary skill in the art would readily accept the suitability of *β-actin* as an internal control gene, Applicant herewith provides a list of dozens of her peer-reviewed studies reporting the successful use of *β-actin* in a large variety of gene expression and tissue applications.

1. Volkenandt, M., Dicker, A.P., Banerjee, D., Fanin, R., Schweitzer, B., Horikoshi, T., Danenberg, K., Danenberg, P. and Bertino, J.R. Quantitation of gene copy number and mRNA using the polymerase chain reaction. Proc. Soc. Exp. Biol. Med., 200: 1-6, 1992.

2. Kan-Mitchell, J., Liggett, P., Taylor, C., Rao, R., Granada, E., Danenberg, K.D., White, W.L., van Zaneldik, L., Horikoshi, T. and Danenberg, P.V. Differential S-100B expression in choroidal and skin melanomas. Invest. Ophthalmol. Vis. Sci. 34: 3366-3375, 1993.

3. Schmittgen, T.D., Danenberg, K.D., Horikoshi, T., Lenz, H.-J. and Danenberg, P.V. Effect of 5-fluoro- and 5-bromouracil substitution on the translation of human thymidylate synthase mRNA. J. Biol Chem., 269: 16269-16275, 1994.

4. Lenz, H.-J., Danenberg, K., Schnieders, B., Goeker, E., Peters, G.J., Garrow, T., Shane, B., Bertino, J.R. and Danenberg, P.V. Quantitative analysis of folylpolyglutamate synthetase gene expression in tumor tissues by the polymerase chain reaction: Marked variation of expression among leukemia patients. Oncology Res., 6: 329-335, 1994.

5. Lenz, H.-J., Hill, C., Danenberg, K.D., Leichman, L.L., Priest, D.G. and Danenberg, P.V. Rapid quantitative PCR for determination of relative gene expressions in tissue specimens. PCR Methods Applic., 4: 305-309, 1995.

6. Johnston, P.G., Lenz, H.-J., Leichman, C.G., Danenberg, K.D., Allegra, C.J., Danenberg, P.V. and Leichman, L. Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. Cancer Res., 55: 1407-1412, 1995.

7. Link, K.H., Kornmann, M., Lenz, H.J., Butzer, U., Pillasch, J., Safi, F., Gansauge, F., Danenberg, K.D., Danenberg, P.V. and Beger, H.G. Response prediction in hepatic artery infusion with fluoropyrimidines using cell culture and thymidylate synthase quantitation. Reg. Cancer Treat., 8: 115-121, 1995.

8. Leichman, L., Lenz, H.-J., Leichman, C.G., Groshen, S., Danenberg, K., Baranda, J., Spears, C.P., Boswell, W., Silberman, H., Ortega, A., Stain, S., Beart, R. and Danenberg, P. Quantitation of intratumoral thymidylate synthase expression predicts for resistance to protracted infusion of 5-fluorouracil and weekly leucovorin in disseminated colorectal

cancers: Preliminary report from an ongoing trial. Eur. J. Cancer., 31A: 1306-1310, 1995.

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